

On the Growth of Bivalve Gills Initiated From a Lobule-Producing Budding Zone

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Abstract. The growth of bivalve gills proceeds at the posterior end of the gill from a meristem-like budding zone, that is, an undifferentiated terminal organ, which continuously proliferates new gill elements in growing bivalves. In representatives of protobranch, filibranch, and eulamellibranch gills (13 species from Protobranchia, Pteriomorpha, Palaeoheterodonta, and Heterodonta), the first growth steps demonstrate a uniform basic pattern. The budding zone produces either transverse folds that split after a transition zone into parallel pairs of lobules (which themselves later differentiate into the inner and outer demibranchs), or it produces the lobules directly, without first forming a transition zone. The lobules elongate, differentiate into lobes, and transform into leaflet-like structures (protobranchs) or into filaments (filibranchs and eulamellibranchs). The filaments represent the differentiated outer margins of each lobe, of which the central tissue (interlamellar septum) becomes incised or fenestrated, or transformed by tissue junctions. A distally located main growth zone for each lobe is suggested. With regard to the delayed onset of the differentiation of the outer demibranch in juvenile unionids, an additional temporary growth zone for filaments is suggested to exist at the anterior end of the outer demibranch.

Introduction

Bivalve gills are unique organs that show a continuous terminal growth by adding new elements in correlation with the lifelong increase in shell size. The gills consist of two plate-like demibranchs that are extended anterior-posteriorly on each side of the visceral mass (the only exception: Lucinidae with only one demibranch; Ridewood, 1903). In the case of the phylogenetically primitive protobranch gill, the demibranchs are comparatively small and consist of a

series of ciliated leaf-like discs. In filibranch gills, the demibranchs are considerably longer and consist of extended parallel structures—the filaments—rather than parallel disks. The filament structure also appears on the surface of the demibranch in eulamellibranch gills; however, their demibranchs are much more complex organs, because the filaments are connected by various tissue junctions (see Ridewood, 1903).

These gills all share two functional elements: a peripheral ciliary pump that creates a flow of oxygenated water over and through the demibranchs, and an internal circulatory system that carries the oxygenated hemolymph to the heart. During the evolution of filibranch and eulamellibranch bivalves, the size of the gills increased in relation to body mass and mantle cavity, and two additional functions evolved: feeding on inhaled particles, facilitated by mucous secretion and followed by food-string transport along food grooves; and in some taxa, brood care within the interlamellar spaces (for reviews, see Purchon, 1968; Bayne *et al.*, 1976; Morton, 1996).

Bivalve gills develop new elements from their posterior end as they grow (Wasserloos, 1911; Ansell, 1962; Kornushin, 1997). In the past, however, studies on gill growth processes focused only on the early organogenesis of the gill during the postlarval development (for review of the older literature, see Raven, 1966). In all subclasses except Protobranchia, these early stages start with a short row of unidirectional slender filaments of only the inner demibranch (Jackson, 1890; Drew, 1901; Wasserloos, 1911; Ansell, 1962; Waller, 1981; Gros *et al.*, 1998; Chaparro *et al.*, 2001). The row of filaments extends in anterior-posterior sequence. Detailed studies of postlarval stages of the eulamellibranch Veneridae revealed that the unidirectional filaments first display knob-like thickenings at their distal ends and then transform to widened, roughly V-shaped filaments; no bending or reflexion was involved in this process (Ansell, 1962; Mouëza *et al.*, 1999). At the end of

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this postlarval development, the new filaments of the inner demibranch arise from the posterior end of the gill base in the form of V-shaped elements (Ansell, 1962).

In contrast to these developmental results on postlarvae and early juveniles, this study is focused on the continual growth of the differentiated bivalve gill from its posterior growth zone, where new filaments are added. Bivalves of different subclasses were examined. For juvenile unionids, we further describe the beginning of the outer demibranch, which lags behind the early formation of the inner demibranch. The results offer new insights into the increase in filament number, the differentiation of the filaments, and bivalve gill growth in general.

Materials and Methods

Material

To examine gill development in bivalves possessing protobranch gills, we studied three *Nucula* species (subclass Protobranchia) preserved in ethanol (Museum Senckenberg, Frankfurt a.M., FRG): *Nucula nucleus* Linnaeus (Helgoland ex Wolf/2, Coll.-No. SMF 320968/2), *N. sulcata* Bronn (Me5/51 Ku/3, Coll.-No. SMF 320966/3), and *N. tenuis* (Montagu) (Gauss-St. 101 Ku/6, Coll.-No. SMF 320967/6).

As examples of filibranch gills, we examined species belonging to the subclass Pteriomorphia: *Mytilus edulis* Linnaeus (sampled at Grömitz on the shore of the Baltic Sea, Schleswig-Holstein, Germany), *M. galloprovincialis* Lamarck (Ria de Vigo, Galicia, Spain), and *Anadara* sp. (Kakinada Bay, Andhra Pradesh, India; soft body directly preserved in Bouin's fluid).

Eulamellibranch gills from the subclasses Palaeoheterodonta [*Unio pictorum* (Linnaeus), *U. tumidus* (Philipsson)] and Heterodonta [*Dreissena polymorpha* (Pallas), *Corbicula fluminea* (O.F. Müller), and *Pisidium casertanum* (Poli)] were studied. *Corbicula* was collected from the Rhine River near Cologne (Rh.-km 683); the other species from waters of the flood plain of the Lower Rhine (Hafensche Landwehr near Rees, Rh.-km 840). We also inspected *Mya arenaria* (L.) from Grömitz and *Venerupis decussata* (L.) from Ria de Vigo.

Scanning electron microscopy

Fresh gills (posterior sections) of *U. pictorum*, *C. fluminea*, *D. polymorpha*, and *P. casertanum* were fixed in 2% glutaraldehyde in 0.133 mol phosphate buffer (pH 7.2) for 2 h. This material, as well as prefixed gills from *Anadara* sp., *N. sulcata*, and *N. tenuis*, were then dehydrated in ethanol. After two rinses in pure acetone for 2 h each, the gills were stored overnight in pure acetone, then dried with CO₂, mounted, and sputtered (ca. 140-nm gold layer).

Histology of subadult *Unio* gills

For histological analysis of the budding zone, two specimens of *U. pictorum* (shell lengths 4.85 mm and 20.1 mm) were fixed in Bouin-Allen's fluid (2 h, 37 °C). After rinsing in 70% ethanol followed by standard dehydration, the tissues were embedded *via* Rotihistol (15 h) and Rotihistol-Rotiplast 1:1 (1.5 h at 61 °C) in Rotiplast (paraffin, melting point 58 °C). Serial 10- μ m microtome sections were stained with Domagk's stain (Romeis, 1968).

Dissection of juvenile gills

Early juvenile eulamellibranchs possess only slender filaments of the inner demibranch. We estimated the shell size at which the outer demibranchs first developed. Specimens of *U. pictorum* and *U. tumidus* (shell length between 3.5 and 16.95 mm) preserved in ethanol were dissected for this purpose. The gills were placed on slides (after dehydration in ethanol) and embedded in Rotihistokitt (Roth, Karlsruhe-FRG). The longest filament of each demibranch was then measured from its dorsal base to its ventral tip with an image analyzing system attached to a CCD-camera linked to a Leitz microscope. With a pointer on the monitor, the length could be measured to the nearest 0.01 mm. Linear regression lines were calculated using SPSS 7.5 and Statgraphics 4.0.

Terms used for gill structures

Various anatomical terms have been used for the description of bivalve gills (Mitsukuri, 1881; Ridewood, 1903; Yonge, 1947; Kiliyas, 1956; Beninger *et al.*, 1988; and others). To avoid terminological confusion, we summarize most of these terms and mark (by single quotation marks and italics) those that we will use in this study. In most aspects we follow Ridewood (1903). However, with regard to the posterior growth zone of the gill, we will introduce new terms.

When juvenile filibranch and eulamellibranch bivalves have passed the early period of gill differentiation, they possess two 'demibranchs' (also gill plates) in an anterior-posterior extension on each side of the foot. These two demibranchs, that is, the 'inner' and 'outer' ones (Fig. 1b, right side: id and od), are attached by a 'gill base' (also gill axis, gill root) on the dorsal side of the 'mantle cavity' (also pallial cavity) between the visceral mass and the mantle. Each dorsoventrally lengthened demibranch is formed into two 'lamella'-like structures (also membrane plates, leaves) consisting of vertically ciliated 'filaments' in parallel (also gill bars, ciliated discs) (Fig. 1a). The terms 'descending limb' of the filament (also descending portion of the filament, *i.e.*, that part of the filament connected to the gill base) and 'ascending limb' (the other part of the filament dorsally unattached or fused with foot or mantle) will be circumvented as far as possible because of developmental

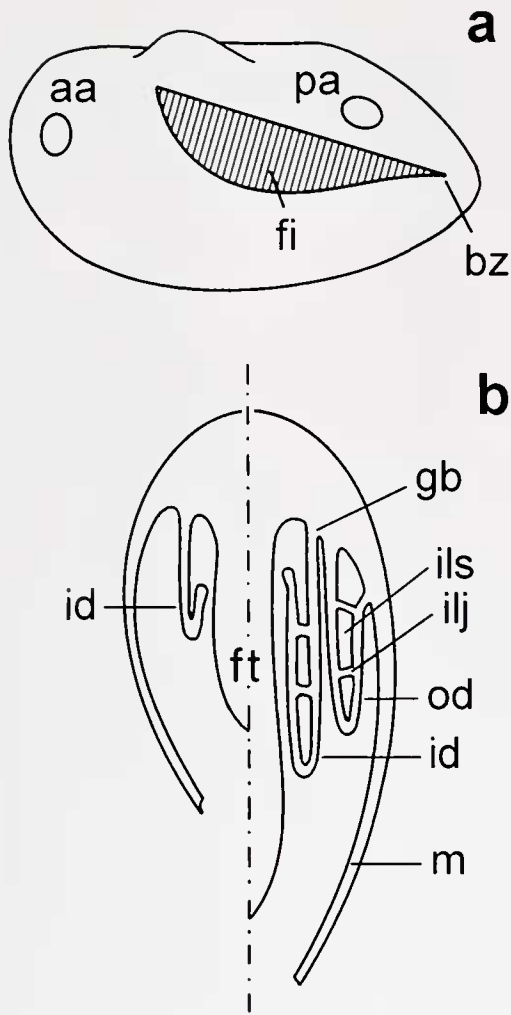


Figure 1. (a) Schematic view of shell shape and the relative position of the two adductor muscles (aa: anterior adductor, pa: posterior adductor) and the gill in *Unio tumidus*. The budding zone (bz) of the successively increasing number of filaments (fi) is located at the posterior end. The filaments lengthen during shell growth. (b) Schematic view of a transverse section showing the age-dependent difference in gill organization between juvenile unionids of shell length 3.5 mm (left half) and 8.1 mm (right half). id: inner demibranch, showing early differentiation and later differentiation on the left and right sides, respectively (in most parts of the gill, not yet attached to the foot), od: outer demibranch, gb: gill base, ilj: interlamellar junction, ils: interlamellar space, m: mantle, ft: foot.

and functional connotations. 'Interlamellar junctions' may stabilize the elongated filibranch filaments, and adjacent filaments are held together by 'ciliated knobs' (also ciliated discs), which are arranged dorsoventrally, and more or less equidistantly (Fig. 2). Eulamellibranchs possess two types of tissue bridges: 'interlamellar junctions' (also septa) between the descending and ascending limbs of the filaments, and 'interfilamentar junctions' between adjacent filaments. The variety of tissue junctions increases the complexity of the branchial architecture, with 'interlamellar spaces' or 'gaps' (also suprabranchial chambers, vertical water tubes, interlamellar cavity) and 'interfilamentar pores' (also ostia,

slits) through which the inhaled water passes. The filaments of each demibranch are strengthened by skeletal rods and are joined at their ventrodorsal margins, thus forming the ciliated 'food groove' (also marginal groove). As the central structures of the lamellae increase in complexity, two general types of gills—homorhabdic and heterorhabdic—become evident in different species. Homorhabdic gills contain only 'ordinary filaments', whereas heterorhabdic gills contain both 'ordinary' and 'principal' filaments (Ridewood, 1903).

Gills of protobranch bivalves are smaller, restricted to the posterior part of the mantle cavity, and characterized by a simple anatomy. However, the gross design is the same as that of the filibranch and eulamellibranch types, that is, two demibranchs on each side. Each consists of a series of extended leaflets (also discs), ciliated and attached to each other by ciliated knobs.

Results

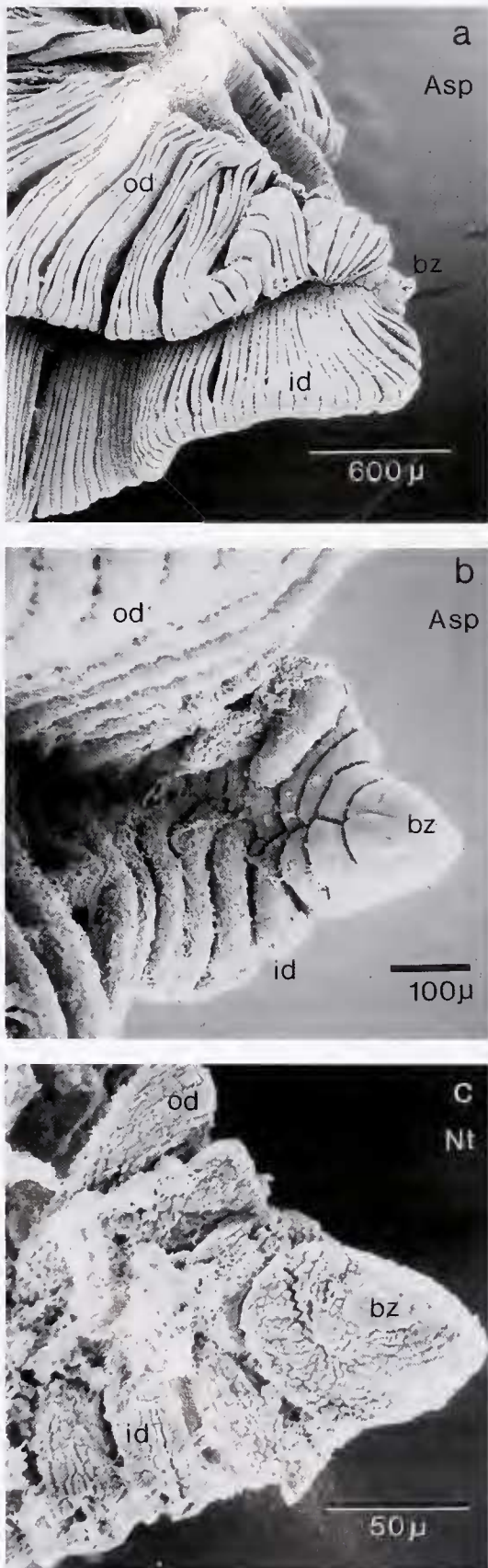
Budding zones of protobranch and filibranch gills

On the basis of our material, the posterior growth zone of these two gill types can be demonstrated best in the filibranch gill of *Anadara* (Pteriomorpha) (Fig. 2a). In this species, the posterior part of the gill base ends in a small, rounded projection of undifferentiated cells, from which the separation of new filaments starts (Fig. 2b). We name this meristem-like cell complex the 'budding zone'.

As was observed in all dissections, the budding zone of *Anadara* is not attached to the mantle but projects into the mantle cavity. The budding zone of the specimen presented (Fig. 2b) is already marked on its ventral side by a fine medial line. This is the onset of the deep longitudinal groove that separates the inner and outer demibranchs. The second step of early differentiation is the appearance of transverse folds which form undifferentiated 'lobules' of the inner and the outer demibranchs in a characteristic 1:1 relationship.

We were able to confirm the 1:1-ratio of demibranch lobules in the filibranch mussel *Mytilus* (not shown). However, the undifferentiated budding zone of *Mytilus* is followed by a 'transition zone' characterized by a number of transverse folds that have not yet split medially into the lobules of the two demibranchs (as in *Unio*, compare Fig. 4a). The length of the transition zone differed among specimens. In *M. galloprovincialis* from Vigo ($n = 12$), small specimens (0.5–0.7 cm) and larger ones (1.0–3.3 cm) revealed 4–6 and 8–12 transverse folds, respectively (exception: one 3.7-cm specimen with only four folds). In *M. edulis* ($n = 7$) from Grömitz, the length of the transition zone had no relation to shell length (2 folds in specimens of 1.7 and 2.1 cm shell length, 5 folds for sizes of 1.8 and 2.1 cm, 7 for a 1.2-cm specimen, 9 and 10 folds for sizes of 3.2 cm and 2.0 cm, respectively). It is possible that such variations are correlated with different rates of gill increase.

Similar to *Anadara*, in *Nucula tenuis* (Protobranchia) the



tiny budding zone of the gill is represented by the posterior apex of the gill base and has no contact to the mantle (Fig. 2c). The same was observed in dissected specimens of the other two *Nucula* species. In *Nucula*, both the transverse folds and the separation of inner and outer demibranchs occur simultaneously. Thus, the lobules appear from the very beginning.

Lobule differentiation in protobranchs and filibranchs

In the protobranch *Nucula*, the lobules of the demi-branches extend laterally and dorsoventrally and form the leaf-like lobes. Part of the margin of each lobe becomes thickened and ciliated, whereas the expanded inner portion of the lobe—its 'lamina' (also interlamellar septum)—remains unchanged. These are then the differentiated leaflets.

The lobules in the filibranchs *Anadara* and *Mytilus* mainly increase dorsoventrally and form elongated lobes. Their margins differentiate into descending and ascending limbs of the filament, as already described for Arcidae and Mytilidae (Ridewood, 1903). Simultaneously, the lamina of each lobe becomes transformed. In *Anadara*, a gap (interlamellar space) occurs within the lamina and separates the filament's margins (Fig. 3a). The length of the gap may be as little as 50% or as much as 90% of the filament's length. The outer margins of the filament now resemble descending and ascending limbs. Adjacent filaments are attached to each other by nearly equidistant ciliated knobs that are arranged in vertical rows, one on either side of the two ciliated margins. Lateral views of the lamellae reveal that the equidistant knobs on adjacent filaments are aligned, and that the number of equidistant lines of knobs increases as the filaments elongate. It was obvious that, during elongation, new lines of knobs appeared stepwise near the filament's distal end. Thus, a main growth zone of each filament must be localized in this distal area (Fig. 3a). However, it can also be seen that a new line of knobs becomes inserted in a few areas along the length of the lamellae after the distance between two rows has increased (not shown). This fact suggests that, in *Anadara* gills, some incremental elongation of the filaments also occurs all along the dorsoventral length of the lamina.

In *Mytilus*, the lobules occur after the medial splitting of the transition zone into inner and outer demibranchs (see above). Each lobule elongates and forms a lobe. Then, its lamina becomes transformed. A few interlamellar junctions

Figure 2. Scanning electron micrographs of the differentiating demi-branches in the filibranch *Anadara* sp. (Asp; full-grown specimen) and the protobranch *Nucula tenuis* (Nt; 6.5 mm). (a) Overview of the posterior end of the left gill of an adult *Anadara* specimen showing the budding zone (bz). (b) Budding zone of *Anadara* differentiating into lobules of the inner and outer demibranch (id, od). (c) Budding zone and separating inner and outer demibranchs of *Nucula*; the lobules are partly covered by mucus and cilia.

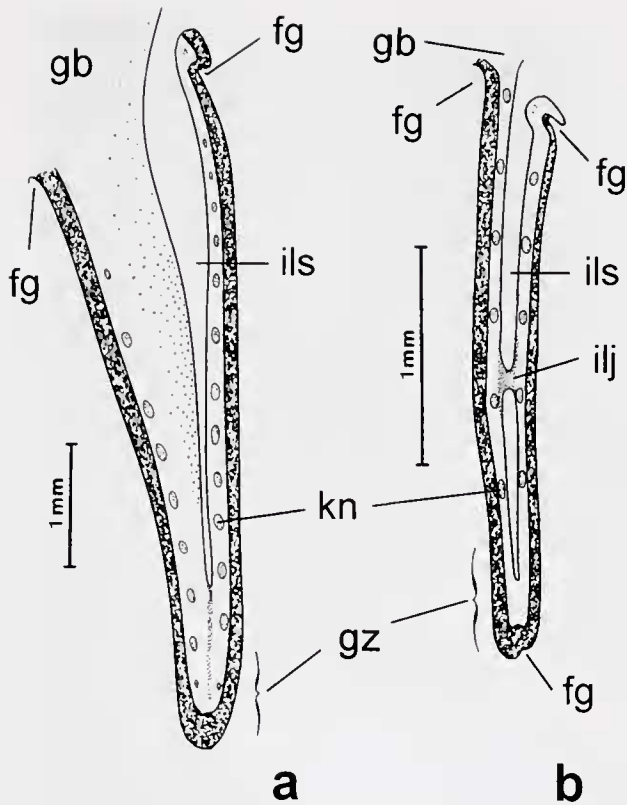


Figure 3. Gill filaments of two filibranch bivalves demonstrating the lengthened lobules with the equidistantly arranged ciliated knobs (kn), which increase in number from the distal end. The proposed distal growth zone (gz) is indicated. (a) *Anadara* sp. (shell size about 5 cm); (b) *Mytilus edulis* (shell size of 2.2 cm). fg: food groove, gb: gill base, ilj: interlamellar junction, ils: interlamellar space.

remain and stabilize the small distance between the outer margins (Fig. 3b); the first ones appeared at about the 30th filament (counted anteriorly from the budding zone). Again, the margins strongly resemble filamentary structures. All adjacent filaments are attached to each other by equidistant ciliated knobs (Fig. 3b). In dissections of the gills of adult *Mytilus*, we observed that the ciliated knobs form equidistant lines, which are arranged parallel to the gill base along the whole gill. The number of lines increased with the size of the demibranchs and the length of their filaments. In contrast to *Anadara*, *Mytilus* shows new lines of knobs only near the ventral end of the filaments. No inserted lines of knobs were detected in lateral views of the lamina. Thus, unlike growth in *Anadara*, the main growth zone responsible for elongation must be restricted to this area.

Budding zones of eulamellibranch gills

The four representatives of the Palaeoheterodonta (*Unio*) and Heterodonta (*Dreissena*, *Corbicula*, and *Pisidium*) studied possess eulamellibranch gills. Again, an undifferentiated budding zone lies at the posterior end of each species' gills (Fig. 4). In each example, the transverse folds form in a way

similar to that of protobranchs and filibranchs: the folds split into inner and outer demibranchs. Apart from these common differentiation events, species-specific differences exist in the relative size of the budding zone and in the extension of a segmented transition zone before the two parallel rows of lobules begin to emerge (Fig. 4). Cilia already occur on the early lobules.

Unio (Fig. 4a): Only the growing left gill and the adjacent mantle epithelium are visible; the right gill is obscured. The budding zone is located on the posterior process of the gill base, which is curved inwards. At least six transverse folds extend from it; this is the transition zone. These folds then become separated by the prospective dorsal food groove into two parallel rows of further enlarged lobules. The lobules become ciliated as they grow ventrally. As can be seen in the outer demibranch (Fig. 4a), the outer edges of the lobules (later forming the outer lamellae) are attached to the mantle tissue from the very beginning. The corresponding processes occur on the other side of the foot, on the growing right gill.

Dreissena (Fig. 4b): In this view from the ventral side, only the left gill is horizontally positioned, so that the details of the transitions between the budding zone and the transverse folds can be seen. The budding zone is smaller than in *Unio* and is followed by a short transition zone. No exceptions to the 1:1 relationship in lobule number between developing inner and outer demibranchs was found. As was demonstrated during dissections, the budding zone is not attached to the mantle.

Corbicula (Fig. 4c): The separation of the transverse folds follows the same principles as described above. The budding zones of the left and right gills lie close together and seem to be attached to the mantle. The dorsal margins of the outer demibranch lobes are also attached to the mantle from the beginning on. However, two peculiarities can be noticed in this species. Firstly, the first transverse fold starts at the inner demibranch of each gill. Secondly, a transition zone, such as is found in *Unio* and *Dreissena*, does not exist.

Pisidium (Fig. 4d): The convergent budding zones were as short as in *Corbicula*. The separation of lobules and their early differentiation appear to be somewhat advanced in the most recent section of the inner demibranch, without disturbing the 1:1 relationship of the older inner and outer lobules (compare idl and odl in Fig. 4d).

Dissection of two other lamellibranchs revealed a differentiation of new lobules more or less identical to that shown in Figure 4. In adult *Venerupis decussata* specimens, neither the budding zone nor the transition zone was fused to the mantle; and the transition zone extended over only two or three transverse folds. When the demibranchs were separated, the most posterior lobules of both demibranchs were still solid, lacking any transformation. A small specimen of *Mya arenaria* (shell length 3.8 mm) showed a similar pat-

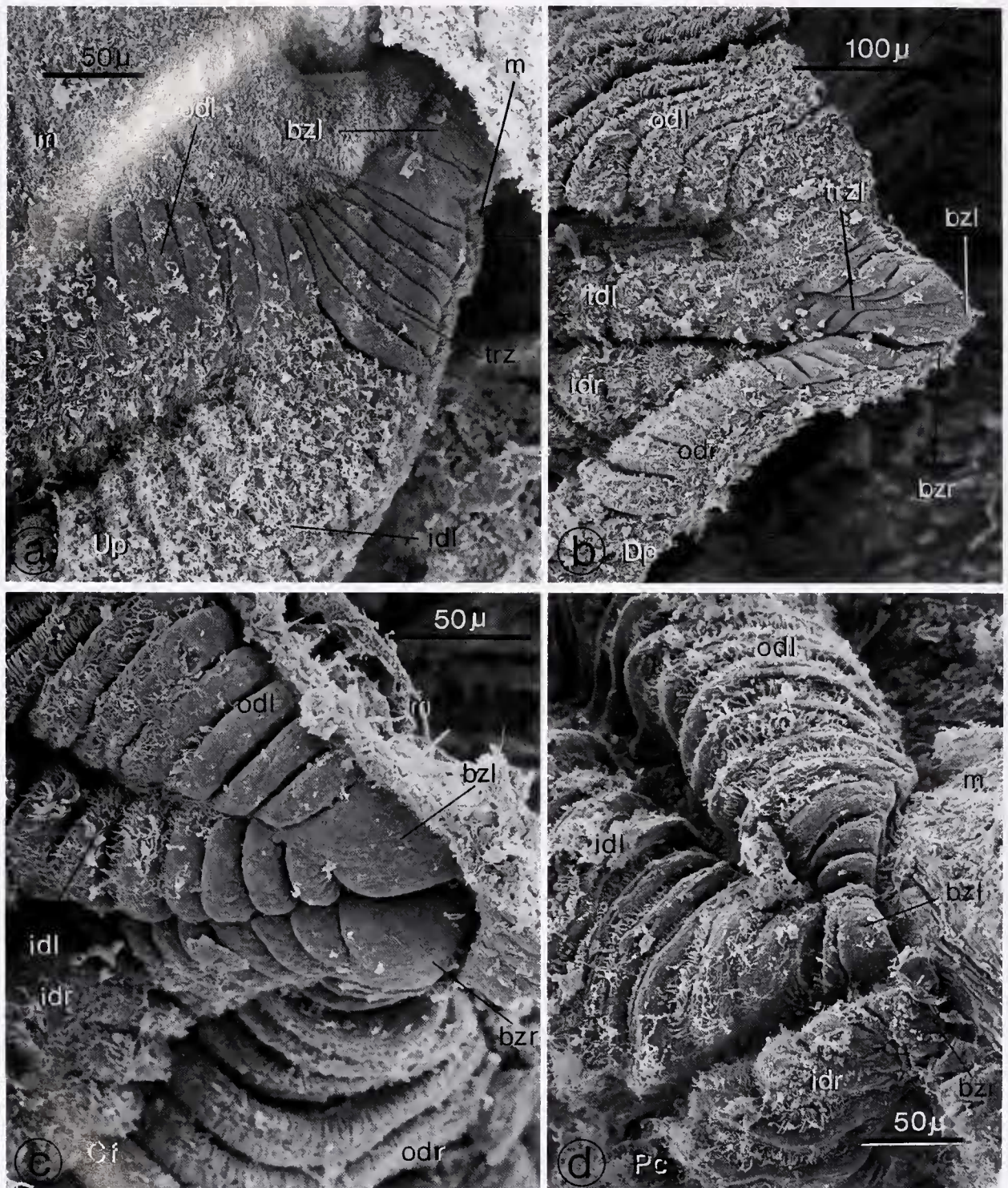


Figure 4. Scanning electron micrographs of the budding zones and the start of both lobule and demibranch differentiation in four eulamellibranch bivalves. (a) *Unio pictorum* (Up; shell length 1.7 cm); (b) *Dreissena polymorpha* (Dp; 1.8 cm); (c) *Corbicula fluminea* (Cf; 0.9 cm); and (d) *Pisidium casertanum* (Pc; 2.1 mm). The budding zones of the left and right gills (bzl, bzl) may lie next to each other, as in Dp, Cf, and Pc. Each is differentiating into the lobules of the separating inner and outer demibranchs (idl, odl, and idr, odr). In *Unio*, a transition zone (trz) of about six transverse folds is shown. m: mantle.

tern. The transition zone consisted of three folds at this stage.

Lobule differentiation in eulamellibranchs

Histological sections of a subadult specimen of *Unio* (Fig. 5) were examined to follow the differentiation of the lobules into the filaments of eulamellibranch gills. The posterior end of the gill, that is, the budding zone, reveals the character of an undifferentiated tissue with a high density of nuclei (Fig. 5a). New transverse folds are added to the already differentiated gill from the budding zone. The longitudinal splitting of the folds into two rows of lobules (representing the inner and outer demibranch) is clearly visible in Figure 5b. The development of these lobules into extended lobes (still unfenestrated) is followed by those differentiations that continuously transform the lengthening lobes into the complexly structured filaments of the eulamellibranchs. Three processes can be distinguished during this development. As they cannot be followed in only one frontal section, we present sections of different levels (Fig. 5a–c).

The first skeletal rods (grayish opaque, no nuclei) are formed in the late transition zone during the beginning of demibranch formation. At first, these rods seem to be confined to the gill base between the inner and outer demibranchs (Fig. 5a); later they extend into the filaments.

The other processes of lobule differentiation can be seen in Figure 5c. Tissue bridges representing interfilament junctions occur between adjacent lobes (ifj in Fig. 5c). As documented further, interlamellar spaces appear in the laminae of most lobes (ils in Fig. 5c). In some lobes, the lamina remains unchanged and constitutes an interlamellar junction (ilj in Fig. 5c). When the demibranchs of eulamellibranchs are observed *in situ*, the lamellae appear filamentous because the outer ciliated margins of the lobes have differentiated into the descending and ascending limbs of the so-called filament. However, this filamentary appearance is due to no more than the outermost 50 μm of the tissue, which bears the ciliary machinery and the vertical hemolymph vessels of the gill.

Outer demibranch formation in juvenile unionids

The dorsal mantle cavity of a 3.5-mm-long specimen of *Unio pictorum* revealed no indication of filaments of the outer demibranch (Fig. 1b). However, six short filaments of the outer demibranch were identified in frontal sections of a *U. pictorum* specimen of 4.9-mm shell length. Apart from the anterior one, these were already differentiated in that they were fused at their outer margins with the mantle epithelium.

Because the development of the outer demibranch lags behind that of the inner, the two demibranchs differ in filament number, in gill-base length, and in demibranch height (as expressed by the length of the longest filament in

the row; Fig. 1a). In Figure 6 the height of the demibranch in juveniles is plotted against shell length, up to 17 mm (years 1–3, based on the number of growth rings). The data for both *Unio* species were pooled because no species-specific deviation was found when they were tested separately ($P > 0.1$). The linear regressions of maximum filament length *versus* shell length were significantly different with respect to the intercepts for the inner and outer demibranchs ($P < 0.001$). The slopes differed only marginally ($P = 0.051$).

In the case of the smallest *Unio* specimen in which an outer demibranch was observed (shell length 4.9 mm), the anteriormost filament of the outer demibranch was located next to the 27th filament of the inner demibranch. In larger specimens, we never found such a large difference in filament number at the anterior margins of inner and outer demibranchs; usually the difference was 10–12 filaments ($n = 10$ specimens with shell lengths between 6.9 and 14.6 mm). Along the whole gill axis, the skeletal rods of parallel filaments of inner and outer demibranchs touched each other at the gill base, resulting in the strict 1:1-arrangement of filaments already shown in Figure 5a.

Discussion

The terminal growth zone of bivalve gills was described based on dissections, scanning electron micrographs, and histological sections. Despite the anatomical differences of the three main gill types (protobranchs, filibranchs, and eulamellibranchs), gill formation in juveniles and adults of 13 species shows a common and uniform pattern. The increase in the number of leaflets in protobranchs, and of filaments in filibranchs and eulamellibranchs, starts from an undifferentiated cell complex that we termed the 'budding zone'. This growth zone generates a series of transverse, paired lobules that constitute, in a 1:1-relationship, both the inner and outer demibranch. The lobules grow into extended and elongated lobes that become transformed into leaflets in protobranch gills and into filaments in filibranch and eulamellibranch gills.

The budding zone should be seen as a specific, undifferentiated complex of dividing cells that is active in growing bivalves. This terminal zone can be characterized as meristem-like because it produces new gill elements during the whole life of these animals, similar to the formation of new leaves from a shoot apical meristem in higher plants, or the development of new polyps from a terminal cell complex in the elongating stems or stolons of thecate hydrozoans (Berking *et al.*, 2002).

One may assume that this terminal growth zone is a projection of the postlarval gill axis composed of peripheral ectodermic and internal mesodermic cells. The budding zone either first produces transversal folds (in cases of delayed splitting into inner and outer demibranch lobules, as in *Mytilus*, *Unio*, *Dreissena*, *Venerupis*, *Mya*) or it directly

forms lobules (in cases of simultaneous splitting of the demibranchs: *Nucula*, *Anadara*, *Corbicula*, *Pisidium*). The segregation may resemble the first steps of somitic segmentation in the early embryology of segmented animals (Wolpert *et al.*, 1998), accompanied by a change in cell adhesion between distinct blocks of ectodermic cells.

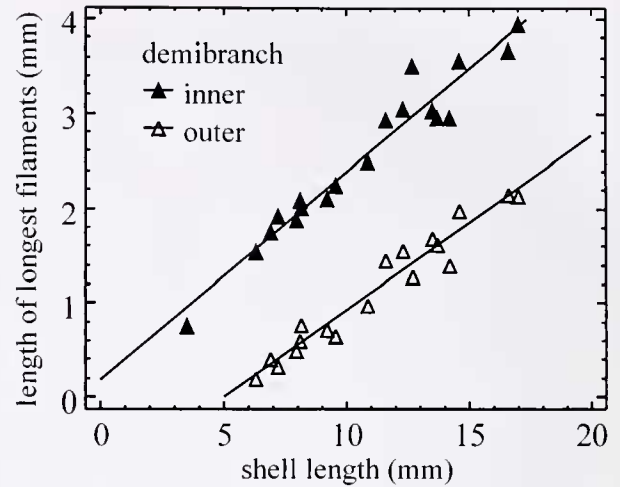
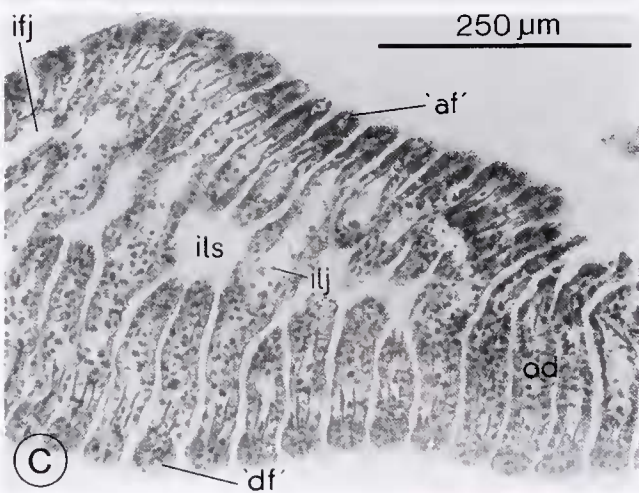
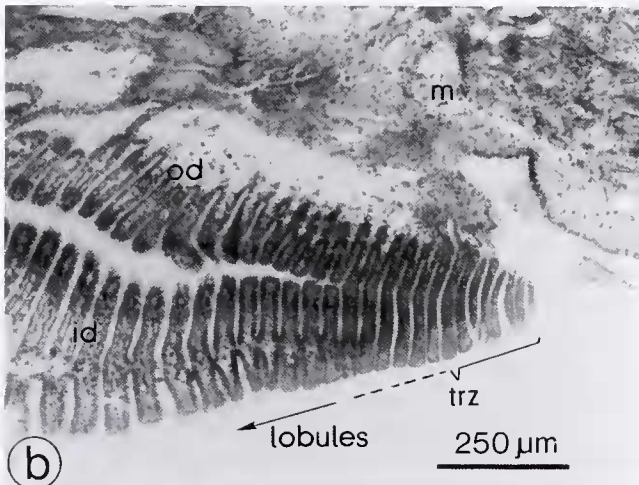
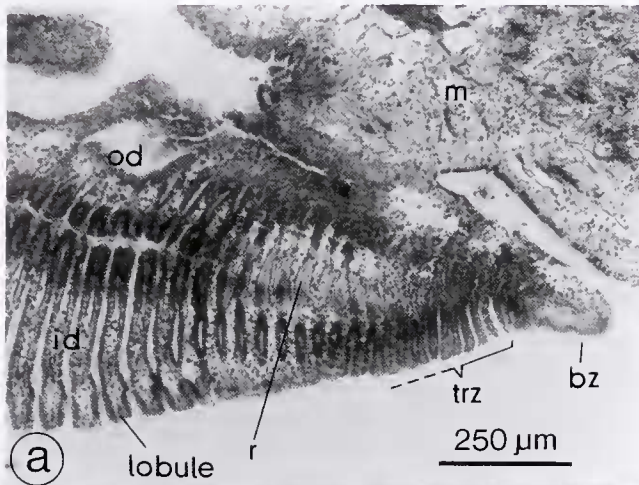


Figure 6. Length of the longest filaments of the inner and outer demibranchs (solid and open triangles, respectively) in *Unio pictorum* and *U. tumidus* specimens of different shell lengths (pooled data of both species). The regression lines are $y_{id} = 0.22(\pm 0.01)x + 0.17(\pm 0.15)$, ($r = 0.97$) in the case of the inner demibranchs; and $y_{od} = 0.19(\pm 0.01)x - 0.93(\pm 0.13)$, ($r = 0.97$) in the case of the outer demibranchs.

The conformity of initial lobule formation in all bivalves tested supports the monophyly of this class. In *Nucula*, the protobranch gill closely resembles the ctenidia of prosobranch gastropods, because both consist of a series of leaflets along a gill axis (Yonge, 1947). This simple gill structure is distinct from the more complex gills in the rest of the bivalves. Based on morphological and molecular data sets, the Protobranchia are therefore considered to be a sister group to the other bivalves, which are grouped as Autobranchia (Hoeh *et al.*, 1998; Giribet and Wheeler, 2002).

In the Autobranchia, the gills are adapted to additional functions, such as feeding and breeding. The decisive evolutionary step was the strong elongation of the lobe. The lobe's transformation into filibranch or eulamellibranch filaments can be understood as a series of developmental steps correlated with increasing efficiencies of the gill's various functions. Fossil records (Cope, 1996) as well as morphological and molecular data (Hoeh *et al.*, 1998; Giribet and Wheeler, 2002) indicate that the filibranch gill represents

Figure 5. Frontal (horizontal) sections (slightly slanted) through the posterior end of the left gill of a subadult *Unio pictorum* (shell-length 20 mm). (a) Section near the gill base with the undifferentiated budding zone (bz), and with a transition zone (trz) and the beginning of lobe differentiation via lobules. (b) The same section (about 30 μm ventral of section a), showing the increasing gap between inner and outer demibranch (id, od). (c) Section of the outer demibranch, ventral to the left part of section b, revealing further details of the differentiation of lobules into early filaments with interfilamentar junctions (ifj), interlamellar spaces (ils), and interlamellar junctions (ilj). 'af': ascending limb of filament, 'df': descending limb of filament, m: mantle tissue, r: rod structure inside the gill base connecting the inner and outer demibranchs.

the plesiomorphic type, and that the eulamellibranch gill characters evolved polyphyletically.

Because lobules and lobes are the primary structural elements, it is interesting to follow their successive transformation into the so-called filaments. The present study confirms that neither bending nor folding of filamentary structures occurs in juvenile and adult bivalves. The final V-shape of the filaments results from the continuous transformation of a lobular anlage *via* lobes into filaments by a dominating ventral growth zone near the tip of the filaments. Evidence of high mitotic activities in this zone was observed in adult filibranchs (*Crenomytilus*, *Mytilus*) by H³-thymidine autoradiographical labeling (Leibson and Movchan, 1975). This result perfectly correlates with our conclusion, which is based on the pattern of equidistant lines of ciliated knobs. Leibson and Movchan (1975) detected two additional areas of DNA-synthesizing activities in *Mytilus*. Both were situated close to the dorsal food grooves, one at the gill base, the other one at the dorsal apex of the filament's ascending limb. As these authors already stated, further studies are needed to investigate whether these two areas represent additional growth zones of the filaments or a higher renewal rate of epithelium cells. In any event, during the transformation of lobes into filaments and the succeeding elongation, no bending or reflection occurs; as declared by Yonge (1947, p. 501): "this mode of origin is impossible." The convenient terms '*descending limb*' and '*ascending limb*' are referring neither to the direction of growth of the filaments, nor to the direction of hemolymph flows, because both arterial and venous lacunae (separated by an intrafilamentar septum; Ridewood, 1903) are located inside the limbs (Yonge, 1947; Kiliyas, 1956; our observations on *Anadara*).

In the early development of the unidirectional slender filaments in postlarvae, developmental processes similar to those described above seem to occur. One could term them '*pro-filaments*' because they strongly differ from the filaments in adults. Based on scanning electron microscopy figures of juveniles of the pseudolamellibranch *Ostrea chilensis* (Chaparro *et al.*, 2001) it may be inferred that growth occurs without bending; the gill rudiments (*e.g.*, pp. 201–203; Fig. 1c, Fig. 2a) perfectly correspond to compact transverse structures, *i.e.*, lobules. During postlarval development of the eulamellibranch Veneridae, the unidirectional filaments of the inner demibranch display a thickened distal end and transform into V-shaped filaments without any bending (Ansell, 1962; Mouëza *et al.*, 1999). This thickened end may be recognized as a kind of lobule. Corresponding conclusions may be derived from the thickened ends of the filaments presented in figures of *Pecten* (Beninger *et al.*, 1994), freshly metamorphosed *Unio* (Herbers, 1913) and juvenile *Sphaerium* (Wasserloos, 1911, figs. K, L).

The onset of the outer demibranch formation and its delay in the Autobranchia reveals two further interesting developmental aspects, as shown in *Unio pictorum* and *U. tu-*

midus. Firstly, a certain body size must be reached before outer demibranch development is initialized. In the *Unio* population we studied, differentiation of the outer demibranch started at shell lengths of about 4–4.5 mm. Specimens of this size showed one growth ring, indicating the cessation of growth during the first winter. In contrast, Korniushin (1997) found the first filaments in 2.4-mm *Unio* specimens. Whether such a difference in the start of outer demibranch formation is due to environmental or genetic factors remains unclear. Secondly, at the anterior end of the gill axis, the number of filaments in inner and outer demibranchs differs. The difference is size-dependent and decreases in larger juveniles. Such a decrease in the difference of anterior filament number was also observed in several other eulamellibranch species (Korniushin, 1997). A reduction of the foremost filaments of the inner demibranch seems to be unlikely, because all filaments were completely differentiated at the 4.9-mm stage; *i.e.*, they were fused to the foot and were functionally integrated. We hypothesize that the outer demibranch also extends its range at its anterior end where—during a short developmental period—a limited number of lobules differentiate from the gill axis in parallel to the filaments of the inner demibranch.

In summary, the generation of simply structured lobules from the posterior budding zone and their differentiation into protobranch leaflets, filibranch filaments (interlamellar junctions and ciliated knobs between adjacent filaments), or more intricate structures (with complex interfilamentar junctions, as in pseudolamellibranchs and eulamellibranchs) may be an interesting model for further developmental studies, which may also offer insight into the evolution of the various gill types that occurred during the phylogeny of bivalves.

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